The efficiency of both pharmacological manipulations of PLC was verified with the DAG-sensor PKCγ-C1 and the PI(4,5)P_2-sensor PLCδ1-PH, monitored in total internal reflection fluorescence microscopy.
These data suggest that activation of PLC is an indispensable step in G_qPCR – TASK signaling and are inconsistent with the hypothesis that direct $G\alpha_q$-interaction mediates TASK current inhibition.
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675-Pos Board B444
Inward Rectification of TWIK-1 Two-Pore Domain K^+ Channels
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Mammalian two-pore domain K^+ channels (K2P) mediate background K^+ conductance and play an important role in regulation of cellular excitability and electrolyte homeostasis. TWIK-1 (also known as K2P1), the first cloned mammalian K2P channel, is highly expressed in the brain, kidney, and heart. They contribute to a large passive K^+ conductance in rat hippocampal astrocytes, conduct inward leak Na^+ currents in human cardiac myocytes under pathological hypokalemia, and regulate phosphate and water transport in mouse proximal tubule and medullary collecting duct, respectively. TWIK-1 K^+ channels were first characterized in Xenopus oocytes and defined as weakly inward rectifying K^+ channels. However, whether TWIK-1 K^+ channels show inward rectification is contradictory, as several reports indicate that TWIK-1 K^+ channels do not exhibit weakly inward rectification when expressed in mammalian cells and Xenopus oocytes. Here we report that TWIK-1 K^+ channels heterologously expressed in Chinese hamster ovary cells show weakly inward rectification in physiological K^+ gradients. Such a rectification is caused by voltage-dependent blockade of intracellular blockers rather than rapid fast inactivation, as intracellular blockers bind to TWIK-1-specific sites in the inner pore of TWIK-1 K^+ channels. These results improve current understandings of the function of TWIK-1 K^+ channels as well as their contributions to cellular behaviors.

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K2P and Kir K^+ Channels in Physiological Bilayers
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We are interested in understanding the mechanism of modulation of inwardly-rectifying (Kir) and two-pore (K2P) potassium ion channels by physiological and modulatory lipids. The phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) is critical for Kir channel activity, and recent crystal structures of Kir2.2 (one of them in complex with PIP_2) have shed light on PIP_2–Kir channel interactions. However, the mechanism of PIP_2 binding and gating is not fully understood. Here, we used a multi-scale approach, consisting of sequential coarse-grained and atomistic molecular dynamics simulations of Kir2.2 embedded in a phospolipid/PIP_2 bilayer, to determine whether this computational approach leads to the same PIP_2–binding site as observed in the PIP_2–bound crystal structure. Our results correctly predict the PIP_2 binding site in Kir2.2 even when slightly different structures were used as starting coordinates for these simulations. These results therefore demonstrate the predictive power of this computational approach for the study of protein interactions with PIP_2 and possibly other modulatory lipids.
Having validated this multi-scale approach, we applied it to study both Kir and K2P channels with PIP_2 in physiological bilayers. TREK-1, the prototypical K2P channel, is modulated by PIP_2, but the exact mechanism by which it interacts with PIP_2 has not been fully elucidated. To explore these mechanisms, we have built structural models of TREK-1 based upon the recent crystal structures of the related K2P channels, TWIK-1 and TRAAK. These two different structural models of TREK-1 were validated by comparison to functional scanning mutagenesis data, which revealed that TRAAK provides the best structural template for modelling of TREK-1. This new structural model of TREK-1 now provides an opportunity to use multi-scale simulations to explore the interaction of TREK-1 with PIP_2/modulatory lipids, and to gain a greater insight into the molecular mechanisms which underlie regulation of K2P channel gating.

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Role of K^+ Channels in Alveolar Macrophages-Mediated Inflammatory Response upon Anthrax Lethal Toxin Stimulation
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The causes of rapid death in anthrax infection both in animals and humans are unknown and concern over the use for biological warfare has renewed interest in elucidating the mechanisms of anthrax induced inflammation. Interleukin-1β (IL-1β) secretion is an important inflammatory response against anthrax lethal toxin (LeTx) a virulence factor of Bacillus anthracis. Here, we report that LeTx induces a significant increase in inwardly-rectifying K^+ (Kir) and voltage-gated K^+ (Kv) currents in mouse and human macrophages. Furthermore, we also show that blocking either Kir or Kv channels significantly inhibits LeTx-induced IL-1β secretion suggesting that activation of macrophage K^+ channels plays an important role in LeTx-induced inflammatory response. In addition, we also investigated the role of macrophage K^+ channels in macrophage priming, a well-known macrophage infection model involving pre-exposure of the cells to a low level of antigen that augments the response to subsequent challenge. Specifically, priming of alveolar macrophages by either Lipopolysaccharides (LPS), an endotoxin of all gram-negative bacteria, or Bacillus spores, augments inflammatory response upon LeTx stimulation as compared to unprimed cells challenged with LeTx alone. Our study shows that pre-exposure to low levels of LPS or to the spores also significantly augments LeTx-induced activation of macrophage K^+ channels suggesting that activation of K^+ channels might be part of the priming mechanism.

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B-Adrenergic Receptor-Mediated Suppression of the Medium After hyperpolarization in Rat Hippocampal Neurons Maintained in Culture
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The firing of a train of action potentials in hippocampal pyramidal CA1 neurons is regulated by both medium (mAHP) and slow (sAHP) afterhyperpolarizations. The mAHP is generated by activation SK and M-channels, and the deactivation of hyperpolarisation-activated H-current. In contrast, the sAHP is mediated by activation of an unknown calcium-dependent potassium channel. Organotypic hippocampal slices are a useful tool for studying the effects of altered protein expression, but it is not known whether neurons maintain the channel subtypes that underlie the mAHP or sAHP. The mAHP (generated by 25 action potentials initiated from a −80 mV) was inhibited 52% by apamin (100 nM) and 49% by XE991 (10 μM), indicating that it is generated by activation of both SK and M channels. The amplitude of the mAHP was found to increase with action potential number, an effect that was blunted by apamin. These data indicate that activation of SK channels, and not M-channels, are primarily responsible for the recruitment of the mAHP in response to increasing trains of action potentials. As previously described, application of isoprenaline significantly suppressed the sAHP. Interestingly, application of isoprenaline (1 μM) also suppressed the mAHP in organotypic slices by 52 ± 0.05 % (n=5). The prior block of SK channels by apamin did not prevent suppression of the mAHP by isoprenaline, indicating that suppression of the mAHP by β-adrenergic receptor activation does not result from inhibition of SK channel activity. These data indicate that organotypic slices retain the channel subtypes that underlie the medium and slow AHPs. The novel effect of isoprenaline suggests that the increased excitability of hippocampal neurons observed in the presence of the β-receptor agonist is a combined effect of suppressing both the medium and slow AHPs.

Voltage-gated Na Channels

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Microsecond Molecular Dynamics Simulations of the Open State Structure of a Bacterial Voltage-Gated Sodium Channel Reveal Mechanisms of Ion Selectivity and Conduction
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Microsecond atomic detail equilibrium molecular dynamics simulations based on the open-state crystal structure (McCusker et al, 2012, Nature Comm) of a bacterial voltage-gated sodium channel (NavMs) have been employed to characterize the mechanisms underlying ion selectivity and conductance of the channel embedded in a lipid bilayer membrane. This approach captured the full plethora of conduction events, revealing a complex mixture of single and multi-ion phenomena, with decoupled rapid bi-directional water transport. Channel selectivity for Na over K ions was found to increase with decreasing applied membrane potential. In marked difference to K-channel simulations, no voltage lag was observed for Na^+. Unlike in K^+ channels, the ions are fully